containing residues 227-236, which exhibits little sequence identity between the various PFKs. Currently Tt(222-242)/LbPFK is being characterized to determine its potential role in PEP binding. The only mutation to show enhancement in PEP binding in LbPFK is D12A (5-fold), which is located on the active site interface approximately 16 Å from the allosteric binding site. The role of D12A is LbPFK is currently under investigation. Funding provided by NIH grant GM33216 and Welch Foundation grant A1548.

204-Pos
Surface-Exposed Hydrophobic Residues on Small Ankyrin-1 Mediate Binding to Obscurin
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Small ankyrin-1 (sAnk1, Ank1.5) is a splice variant of the ANK1 gene that binds to the large modular protein, obscurin A, with nanomolar affinity, a reaction that may help to organize the sarcoplasmic reticulum in striated muscle. A subset of lysine and arginine residues in the 2 ankyrin repeats of sAnk1 interact specifically with 4 glutamate residues in a stretch of 30 amino acids of obscurin to mediate binding. Homology modeling and molecular dynamics simulations have revealed a ''hot spot'' of 4 hydrophobic residues exposed on the surface of the ankyrin repeat domain of sAnk1. We used site-directed mutagenesis of bacterially expressed fusion proteins, followed by blot overlays and surface plasmon resonance assays, to study the contribution of these 4 residues, V70, F71, I102 and I103, to binding to the 30-mer of obscurin. Alanine mutations of each of these four residues inhibited binding to residues 6316-6345 of obscurin ($Obsc_{6316-6345}$). In contrast, V70A and I102A mutations had no effect on binding to a second sAnk1 binding site on obscurin, located within residues 6231-6260 ($Obsc_{6231-6260}$). Using the same methods, we mutated the 5 hydrophobic residues present in $Obsc_{6316-6345}$ to alanine and identified V6328, I6332, and V6334 as critical for proper binding. Our results suggest that hydrophobic interactions as well as electrostatic interactions are important for the binding of sAnk1 to $Obsc_{6316-6345}$, consistent with studies of the complexes formed by other ankyrin repeat proteins with their ligands. Hydrophobic interactions are likely to contribute to the difference in affinity of sAnk1 for $Obsc_{6316-6345}$ and $Obsc_{6231-6260}$, and for the dominant role played by the more C-terminal sequence in binding.
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205-Pos
Clustering Method in QMMM Modeling of the HLADH Binding Site
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Some of the recent advances in quantum mechanical molecular mechanics (QMMM) related to work done on bio-molecular clusters are presented. The main framework of the discussion is related to the interface as made available in GROMACS, but also includes improvements of the same as well a newly developed clustering method and an interface code for the Massively Parallel Quantum Chemistry (MPQC) suite. The clustering method implemented provides an efficient means of studying systems undergoing large scale fragmentation processes where the Quantum Mechanical (QM) region is effectively split or large systems with several separate QM sites. Some aspects of the QMMM code will be presented as well as preliminary results from recent studies on the Horse Liver Alcohol Dehydrogenase (HLADH) binding site.
Keywords: QMMM, Clustering, ADH, GROMACS, MPQC.

206-Pos
Effects of KCL on Calmodulin Mutants Defective in Ion Channel Regulation
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Calmodulin (CaM) is an essential eukaryotic calcium sensor that regulates many ion channels and enzymes. CaM is comprised of two homologous domains (N and C), each with two calcium-binding sites. *Paramecium* mutants identified by a genetic screen to be defective in response to external stimuli showed that the two domains of CaM have different effects on ion channel regulation. Under-reactive mutants (changes in the N-domain of CaM) affect regulation of a calcium-dependent Na^+ current, while over-reactive mutants (changes within the C-domain) affect a calcium-induced K^+ current. Because CaM binds to the intracellular regions of these channels, it is subject to changing concentrations of Na^+ and K^+. This study explores the effects of potassium on the domain-specific conformation and calcium-binding energetics of under- and over-reactive mutants. Potassium-induced changes in altered thermal stability of apo (calcium-depleted) CaM explored effects on tertiary structure. Fluorescence-monitored calcium titrations over the range of 0 to 300 mM KCl showed that the total free energy of binding calcium to each domain became less favorable by about 2.5 kcal/mol. In thermal denaturation studies of apo PCaM, the melting temperature (T_m) increased by approximately 5°C and the enthalpy (ΔH) changed by 3.5 kcal/mol when [KCl] increased from 50 to 300 mM. The findings indicate that potassium ions increased tertiary constraints on apo CaM, making it less flexible. Linkage relationships resulted in lowering calcium-binding affinity. Thus, an influx of K^+ through an ion channel would shift the equilibrium of CaM towards the apo state. This effect would be exacerbated for over-reactive mutants that have intrinsically lower calcium affinity than wild-type CaM.

207-Pos
Redefining the Role of the Quaternary Shift in the Allosteric Inhibition of Bacillus Stearothermophilus Phosphofructokinase
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Bacillus stearothermophilus PFK (BsPFK) is a homotetramer that is allosterically inhibited by phosphoenolpyruvate (PEP), which binds along one dimer-dimer interface. The substrate, fructose-6-phosphate (Fru-6-P), binds along the other dimer-dimer interface. The inhibitor-bound structure compared to the substrate-bound structure of wild-type BsPFK exhibits a 7° rotation about the substrate binding interface, termed the quaternary shift. Evans, et. al. proposed that the quaternary shift is the mechanism for allosteric inhibition for BsPFK. However, the main role of the quaternary shift may be in ligand binding and not allosteric inhibition. The variant D12A BsPFK shows a 100-fold increase in the binding affinity for PEP, a 50-fold decrease in the binding affinity for Fru-6-P, and a coupling comparable to wild-type. Crystal structures of apo and PEP bound forms of D12A BsPFK both indicate a shifted structure similar to the inhibitor-bound structure of wild-type. Remarkably, D12 does not directly bind to either substrate or inhibitor, and is located along the substrate binding interface. A conserved hydrogen bond between D12 and T156 takes place across the substrate binding interface in the substrate-bound form of BsPFK. The variant T156A BsPFK, when compared to wild-type, shows a 30-fold increase in PEP binding affinity, a 17-fold decrease in Fru-6-P binding affinity, and an estimated coupling that is at least wild-type coupling. In addition, T156A BsPFK crystal structure exhibits a shifted structure similar to D12A BsPFK and the inhibitor-bound structure of wild-type. PEP still inhibits these variants of BsPFK despite the fact that the enzymes are in the quaternary shifted position prior to PEP binding. Therefore the quaternary shift of BsPFK primarily perturbs ligand binding but does not directly contribute to heterotropic allosteric inhibition. Supported by NIH Grant GM33216 and Welch Foundation Grant A1548.

208-Pos
Computational Studies of Evolutionary Selection Pressure on Rainbow Trout Estrogen Receptors
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Molecular dynamics simulations were used to determine the binding affinities between the hormone 17β-estradiol (E2) and different estrogen receptor (ER) isoforms in the rainbow trout (*Oncorhynchus mykiss*). Previous phylogenetic analysis demonstrated that a recent, unique gene duplication of the ERα subtype created two isoforms ERα1 and ERα2, and an early secondary split of ERβ produced two distinct isoforms ERβ1 and ERβ2. The objective of our computational studies is to provide insight into the underlying evolutionary selection pressure on the ER isoforms. For the α subtype our results show that E2 binds preferentially to ERα1 over ERα2. In addition, based on the phylogenetic analysis ERα2 should be free from selective pressure and accumulated a considerable amount of mutations. These results suggest that the presence of ERα2 in the genome and its lower binding affinity exhibits, at least, no deleterious effects to its host organism. For the β subtype, both isoforms bind competitively to E2. The strong binding affinity of ERβ2 suggests that the second isoform is likely on the verge of functional specialization and cannot be substituted by the first isoform.

209-Pos
Use of Crystal MD Simulations to Speed Up Evaluation of Binding Free Energies of Dimannose Deoxy Analogs With M4-P51g-Cyanovirin-N
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Molecular Dynamics (MD) presents an advanced tool for scoring of the binding free energies (ΔG) between a target protein and a set of candidate substrates, narrowed by extensive virtual screening process. Molecular mechanics(MM)/continuum model approach for evaluation of ΔG includes calculation of (i) a critically important solvation energy electrostatic contribution by means of solving the Poisson-Boltzmann (PB) or generalized Born (GB) equation and (ii) nonpolar component estimated from the solvent accessible area (SA) of solutes. Both, MM/PBSA and MM/GBSA, methods imply averaging of ΔG over a set of snapshots generated through, preferably, explicit solvent MD